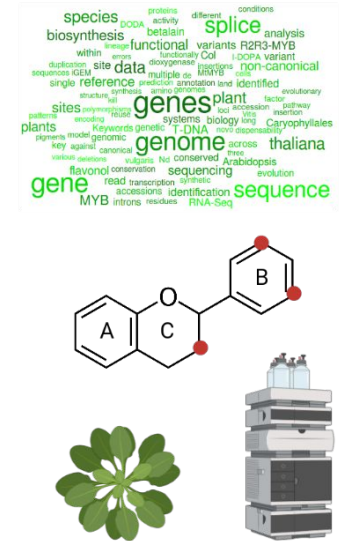
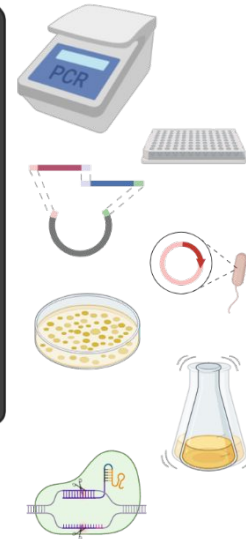
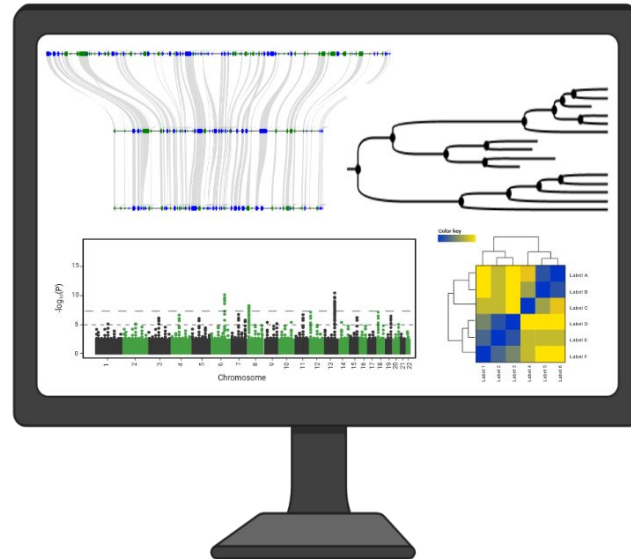
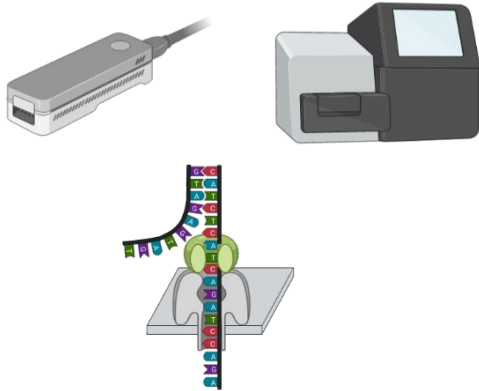




Technische
Universität
Braunschweig



Plant Biotechnology
and Bioinformatics

Python - Application examples

Prof. Dr. Boas Pucker (Plant Biotechnology and Bioinformatics)

Availability of slides

- All materials are freely available (CC BY) - after the lectures:
 - StudIP: 'Python for Life Scientists'
 - GitHub: <https://github.com/bpucker/teaching>
- Questions: Feel free to ask at any time
- Feedback, comments, or questions: [b.pucker\[a\]tu-bs.de](mailto:b.pucker[a]tu-bs.de)

My figures and content can be re-used in accordance with CC BY 4.0, but this might not apply to all images/logos. Some figure were constructed using bioRender.com.

Reverse complement of nucleotide sequence

What happens here?

```
1 def revcomp( seq ):
2
3     seq = seq.lower()
4
5     #key:value (=dictionary)
6     complement = { 'a':'t', 't':'a', 'c':'g', 'g':'c' }
7
8     new_seq = []
9
10    for nt in seq:
11        new_seq.append( complement[ nt ] )
12
13    #list[::-1] inverts list (last element becomes first)
14    new_seq = "".join( new_seq[::-1] )
15
16    return new_seq
```

Sequence of bases e.g. ATGACATGA

Converts input to lower case: atgacatga

Get complement for each base

Inverts list (=reverse)

How to use dictionaries

- Values are accessible via keys
- Keys need to be unique
- Quick access to data based on key
- Higher memory occupation than lists/strings

```
my_dict = {"k1": "v1", "k2": {"x1": "y1"}, 5: ["one", "two", "three"], "hello world": "hello world" }  
print(my_dict.keys()) #all keys of a dictionary  
print(my_dict.values()) #all values of a dictionary  
print(my_dict["k1"])  
print(my_dict["k2"]["x1"])
```

```
dict_keys(['k1', 'k2', 5, 'hello world'])  
dict_values(['v1', {'x1': 'y1'}, ['one', 'two', 'three'], 'hello world'])  
v1  
y1
```

Exercises - Part 6a

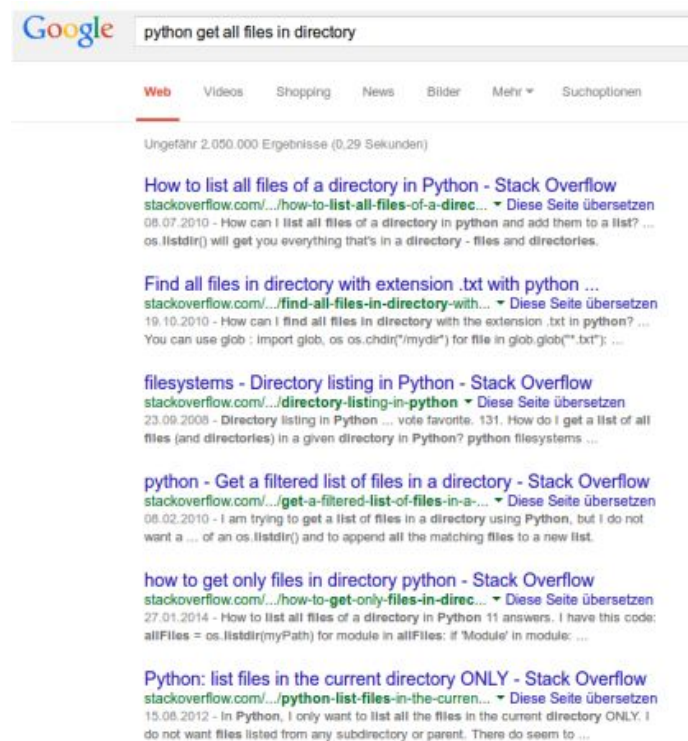
- 6.1) Write a function to get the reverse complement (upper case letters) of a DNA sequence given in upper case letters!
- 6.2) Write a function to convert a DNA sequence into a RNA sequence!
- 6.3) Write a function to translate a DNA sequence into amino acids (first frame only)!
- 6.4) Write a function to translate DNA sequences in all 6 frames into peptide sequences! The longest peptide sequence per DNA sequence should be returned!
- 6.5) Write a function to grep a sequence from a FASTA file based on the name of this sequence!

How to approach a challenge in bioinformatics?

- Identify the problem that needs to be solved
- Split the problem into smallest possible parts
- Solutions for small parts of the problem might be available already
- Precise description of the problem is required for online search
- Best hit often leads to stackoverflow:
 - Problem is described by the asking person
 - Multiple solutions are suggested by the community
 - Community votes to identify the best solution
 - Green marking highlights the answer that solved the problem

Example

- Problem: get paths of all files in a certain directory
- Search expression: 'python get all files in directory'



Best hit on stackoverflow

533 user like
this answer

This answers
solved the
question

14 Answers

active oldest votes

▲
533
▼

You can use `glob`:

```
import glob, os
os.chdir("/mydir")
for file in glob.glob("*.txt"):
    print(file)
```

or simply `os.listdir`:

```
import os
for file in os.listdir("/mydir"):
    if file.endswith(".txt"):
        print(file)
```

or if you want to traverse directory, use `os.walk`:

```
import os
for root, dirs, files in os.walk("/mydir"):
    for file in files:
        if file.endswith(".txt"):
            print(os.path.join(root, file))
```

share improve this answer

edited Apr 22 at 17:44

 Tarantula
4,794 ● 3 ● 22 ● 39

answered Oct 19 '10 at 1:12

 ghostdog74
102k ● 17 ● 116 ● 188

1 Using solution #2, How would you create a file or list with that info? – Merlin Oct 19 '10 at 3:46

35 @ghostdog74: In my opinion it would more appropriate to write `for file in f` than `for files in f` since what is in the variable is a single filename. Even better would be to change the `f` to `files` and then the for loops could become `for file in files`. – martineau Oct 26 '10 at 14:18

18 @computermacygyver: No, `file` is not a reserved word, just the name of a predefined function, so it's quite possible to use it as a variable name in your own code. Although it's true that generally one should avoid collisions like that, `file` is a special case because there's hardly ever any need to use it, so it is often consider an exception to the guideline. If you don't want to do that, PEP8 recommends appending a single underscore to such names, i.e. `file_`, which you'd have to agree is still quite readable. – martineau Oct 14 '12 at 19:04

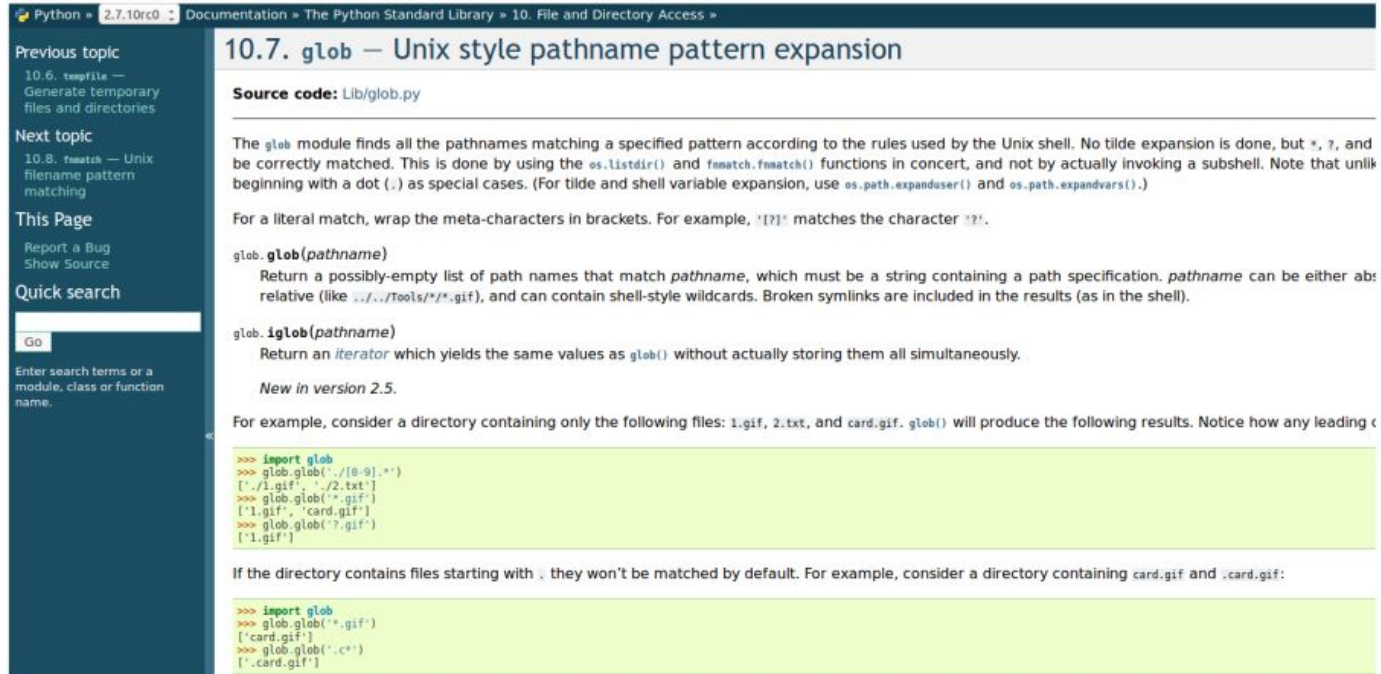
2 Thanks, martineau, you're absolutely right. I jumped too quickly to conclusions. – computermacygyver Oct 15 '12 at 19:53

1 Really cool answer, you could replace `r,d,f` by `r_,d_,f_` to avoid unused variable declaration. – AsTeR Mar 8 '13 at 20:16

Three different
ways to solve this
problem are
described

Official Python documentation

- Systematic documentation of all functions in a module with all possible arguments
- Sometimes examples are given



The screenshot shows the official Python documentation for the `glob` module. The page title is "10.7. glob — Unix style pathname pattern expansion". The left sidebar contains navigation links for "Previous topic" (10.6. `tempfile`), "Next topic" (10.8. `fnmatch`), "This Page" (Report a Bug, Show Source), and "Quick search" (Go). The main content area includes the "Source code: Lib/glob.py" link, a description of the `glob` module, and examples of its usage.

10.7. glob — Unix style pathname pattern expansion

Source code: [Lib/glob.py](#)

The `glob` module finds all the pathnames matching a specified pattern according to the rules used by the Unix shell. No tilde expansion is done, but `*`, `?`, and `[]` are correctly matched. This is done by using the `os.listdir()` and `fnmatch.fnmatch()` functions in concert, and not by actually invoking a subshell. Note that unlike `fnmatch`, `glob` does not begin matching with a dot (`.`) as special cases. (For tilde and shell variable expansion, use `os.path.expanduser()` and `os.path.expandvars()`.)

For a literal match, wrap the meta-characters in brackets. For example, `'[?]'` matches the character `'?'`.

glob.glob(pathname)
Return a possibly-empty list of path names that match *pathname*, which must be a string containing a path specification. *pathname* can be either absolute or relative (like `../Tools/*/*.gif`), and can contain shell-style wildcards. Broken symlinks are included in the results (as in the shell).

glob.iglob(pathname)
Return an *iterator* which yields the same values as `glob()` without actually storing them all simultaneously.

New in version 2.5.

For example, consider a directory containing only the following files: `1.gif`, `2.txt`, and `card.gif`. `glob()` will produce the following results. Notice how any leading `.` is required:

```
>>> import glob
>>> glob.glob('./[0-9].*')
['1.gif', '2.txt']
>>> glob.glob('*card.gif')
['1.gif', 'card.gif']
>>> glob.glob('?.gif')
['1.gif']
```

If the directory contains files starting with `.` they won't be matched by default. For example, consider a directory containing `card.gif` and `.card.gif`:

```
>>> import glob
>>> glob.glob('*card.gif')
['card.gif']
>>> glob.glob('.*card.gif')
['.card.gif']
```

How to run BLAST?

- Running at the NCBI website does not give you full control over all parameters

- Python can be used to run a local search:

```
blastn \
```

```
-query <query_file> \
```

```
-subject <subject_file> \
```

```
-out <output_file> \
```

```
-outfmt 6 \
```

```
-evalue 0.01 \
```

```
-word_size 4
```

1.	qseqid	query (e.g., gene) sequence id
2.	sseqid	subject (e.g., reference genome) sequence id
3.	pident	percentage of identical matches
4.	length	alignment length
5.	mismatch	number of mismatches
6.	gapopen	number of gap openings
7.	qstart	start of alignment in query
8.	qend	end of alignment in query
9.	sstart	start of alignment in subject
10.	send	end of alignment in subject
11.	evalue	expect value
12.	bitscore	bit score

<https://www.metagenomics.wiki/tools/blast/blastn-output-format-6>

How to execute processes via shell?

- Running shell commands through the subprocess module:
p = subprocess.Popen(arg='ls -lh', shell=True)
p.communicate()
- Can be used to run everything via Python
- Python waits until the command is completed
- Example:

```
import subprocess  
p = subprocess.Popen( arg="mkdir test && cd test && ls -lh", shell=True )  
p.communicate()
```

How to process BLAST results?

```

1 def load_BLAST_results( input_file ):
2     """! @brief load all BLAST results from file """
3
4     data = []
5     with open( input_file, "r" ) as f:
6         line = f.readline()
7         while line:
8             parts = line.strip().split('\t')
9             data.append( { 'query': parts[0],
10                          'subject': parts[1],
11                          'query_start': int( parts[6] ),
12                          'query_end': int( parts[7] ),
13                          'score': float( parts[-1] )
14                        } )
15             line = f.readline()
16     return data

```

AT1G01010	NdCChr1.g1.t1	100.00	429	0	0	1	429	1	429	0.0	895	
AT1G01010	NdCChr4.g18734.t1		32.84	469	247	15	1	428	2	443	4e-56	194
AT1G01010	NdCChr1.g127.t1	34.23	336	157	10	1	330	1	278	1e-41	152	
AT1G01010	NdCChr1.g128.t1	32.38	349	157	14	1	331	1	288	4e-39	146	
AT1G01010	NdCChr4.g18730.t1		39.33	178	95	5	1	175	2	169	1e-32	126
AT1G01010	NdCChr3.g12773.t1		39.63	164	89	2	1	162	1	156	1e-28	115
AT1G01010	NdCChr4.g22969.t1		40.74	162	79	5	5	159	11	162	3e-28	117
AT1G01010	NdCChr4.g18733.t1		40.00	165	90	5	1	162	2	160	4e-28	115
AT1G01010	NdCChr3.g17122.t1		42.31	156	74	6	5	153	15	161	4e-27	112

How to organize a Python script

- Make a script recognize that it needs to run with Python:

`#!/usr/bin/env python3`

- Other information to include:

- Author
- Version
- Usage
- Imports

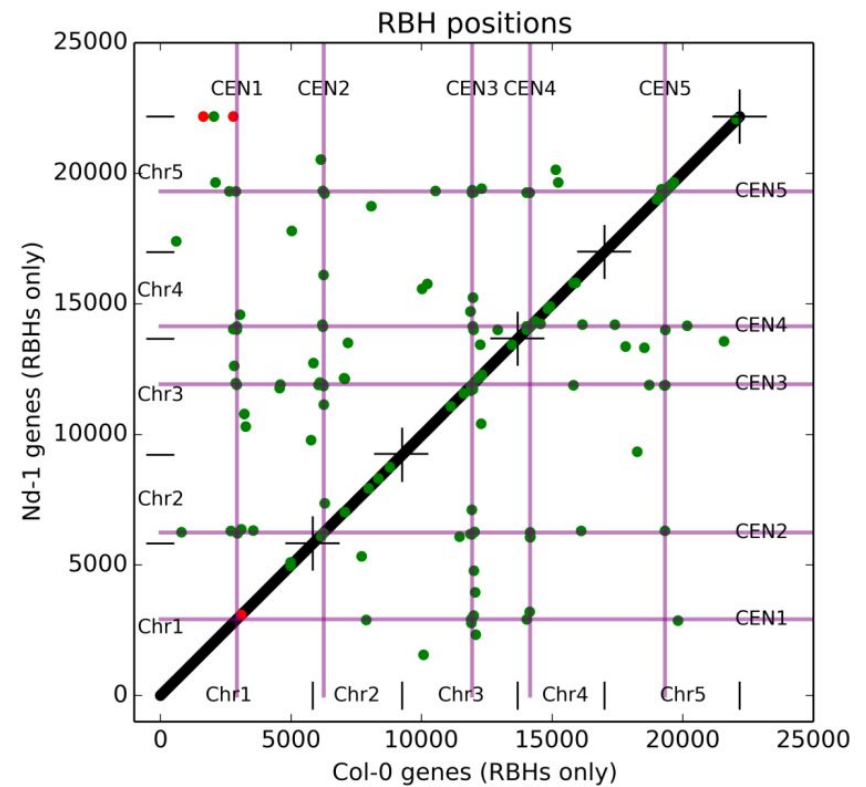
```
1  ### Boas Pucker ###
2  ### bpucker@cebitec.uni-bielefeld.de ###
3  ### v0.2 ###
4
5  _usage_ = """
6      python construct_RNA_seq_coverage_file.py\n
7      --in <BAM_FILE>
8      --out <OUTPUT_FILE>
9
10     --bam_is_sorted <PREVENTS_EXTRA_SORTING_OF_BAM_FILE>
11
12     feature requests and bug reports: bpucker@cebitec.uni-bielefeld.de
13     """
14
15  _cite_ = """ Pucker & Brockington, 2018: https://doi.org/10.1186/s12864-018-5360-z """
16
17
18  import os, sys
19
20  # --- end of imports --- #
21
22  def main( arguments ):
```

How to pass arguments to a Python script?

```
1
2 __usage__ = """ how to run the script and list of arguments """
3
4 def main( arguments ):
5     """! @brief run everything """
6
7     fasta_file = arguments[ arguments.index( '--fasta' )+1 ]
8     gff3_file = arguments[ arguments.index( '--gff3' )+1 ]
9     species = arguments[ arguments.index( '--species' )+1 ]
10    output_dir = arguments[ arguments.index( '--tmp' )+1 ]
11    hints_file = arguments[ arguments.index( '--hints' )+1 ]
12
13    if '--cutoff' in arguments:
14        cutoff = int( arguments[ arguments.index( '--cutoff' )+1 ] )
15    else:
16        cutoff = 1
17
18    #everything happens here
19
20    if '--fasta' in sys.argv and '--gff3' in sys.argv and '--species' in sys.argv and '--tmp' in sys.argv and '--hints' in sys.argv:
21        main( sys.argv )
22    else:
23        sys.exit( __usage__ )
```

matplotlib

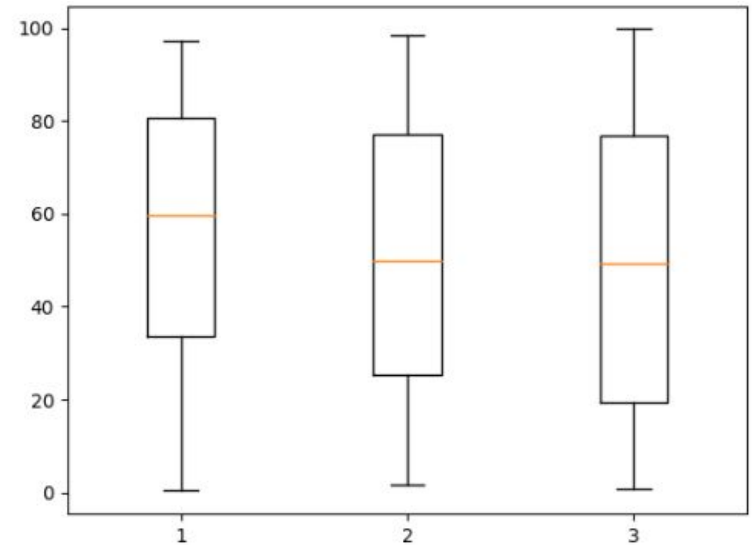
- Importing matplotlib:
`import matplotlib.pyplot as plt`
- Visualization of complex data
- Automatic generation of plots
- Unlimited customization options



Pucker et al., 2016: <https://doi.org/10.1371/journal.pone.0164321>

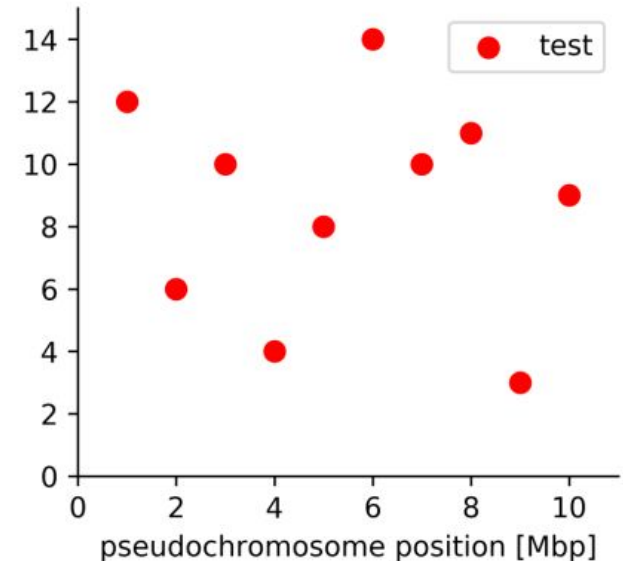
Box plot

```
1 import matplotlib.pyplot as plt
2 import numpy as np
3
4 d1 = np.random.rand(50) * 100 #generate random numbers
5 d2 = np.random.rand(50) * 100
6 d3 = np.random.rand(50) * 100
7
8 data = [d1, d2, d3] # multiple box plots on one figure
9
10 plt.figure()
11 plt.boxplot(data)
12 plt.show()
```



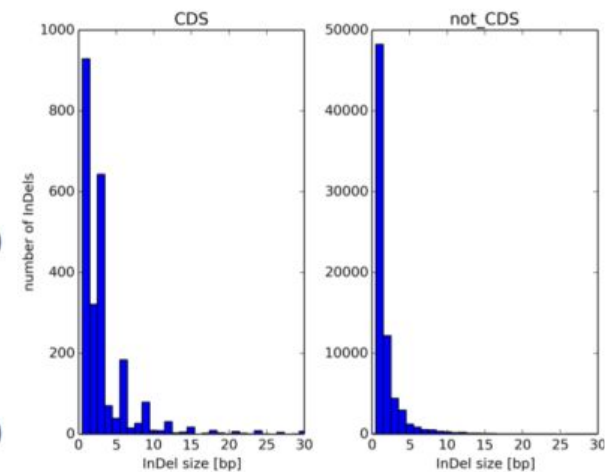
Scatter plot

```
1 import matplotlib.pyplot as plt
2
3 fig, ax = plt.subplots( figsize=( 10, 4 ) ) #defining size of plot
4
5 x_values = [ 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 ]
6 y_values = [ 12, 6, 10, 4, 8, 15, 10, 11, 3, 9 ]
7
8 ax.scatter( x_values, y_values, color="red", s=10, marker="o", label="test" )
9 #setting color, marker size, marker shape and label of this group
10
11 ax.legend( numpoints=1 )
12 #each group is represented by only one marker in the legend (default=3)
13
14 ax.set_xlim( 0, 11 ) #set range of x-axis
15 ax.set_ylim( 0, 15 ) #set range of y-axis
16
17 ax.set_xlabel( "pseudochromosome position [Mbp]" )
18
19 ax.spines["top"].set_visible(False) #remove lines and ticks
20 ax.spines["right"].set_visible(False) #remove lines and ticks
21
22 plt.subplots_adjust(left=0.05, right=0.99, top=0.97, bottom=0.12)
23 #adjust size of plot within figure
24
25 plt.show()
26 fig.savefig( "my_plot.png", dpi=600 ) #write figure into output file
27 plt.close( "all" ) #destroy created figures (cleaning up)
```



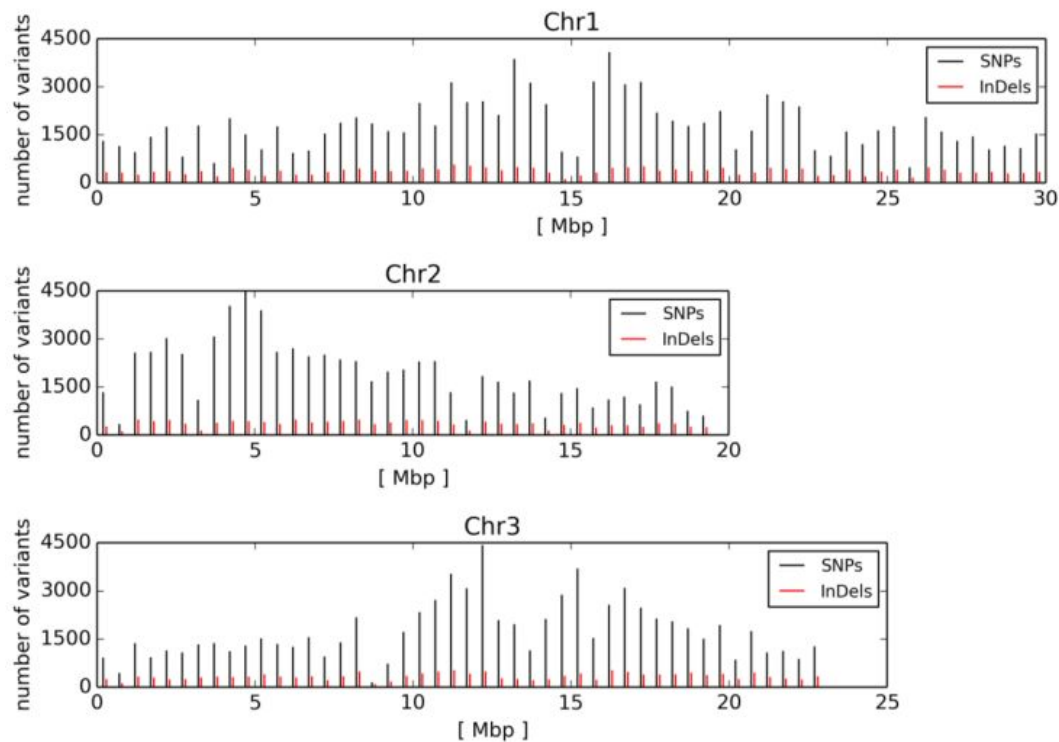
Histogram

```
1 import matplotlib.pyplot as plt
2
3 # --- end of imports --- #
4
5 gene_space = [ 3, 3, 6, 6, 9, 9, 12, 3, 3, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10,
6               11, 12, 13, 14, 15, 16, 17, 18, 19, 20,
7               12, 15, 18, 21, 24, 27, 30 ]
8 intergenic = [ 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1, 2, 3, 4, 5, 6, 7, 8, 9,
9               1, 2, 3, 4, 5, 6, 7, 1, 2, 3, 4, 1, 2, 1 ]
10
11
12 fig, ( ax1, ax2 ) = plt.subplots( 1, 2, sharey=False)
13 counts, bins, patches = ax1.hist( gene_space, bins=max( gene_space ), align="left" )
14 ax1.set_title( "CDS" )
15 ax1.set_xlim( 0, 30 )
16 ax1.set_xlabel( "InDel size [bp]" )
17 ax1.set_ylabel( "number of InDels" )
18
19 counts, bins, patches = ax2.hist( intergenic, bins=max( intergenic ), align="left" )
20 ax2.set_title( "not CDS" )
21 ax2.set_xlim( 0, 30 )
22 ax2.set_xlabel( "InDel size [bp]" )
23 plt.subplots_adjust( wspace=0.3 ) #increase space between figures
24
25 plt.show()
26 fig.savefig( prefix + "InDel_size_distribution.png", dpi=300 )
27 plt.close('all')
```



Pucker et al., 2016: <https://doi.org/10.1371/journal.pone.0164321>

Barplot figure

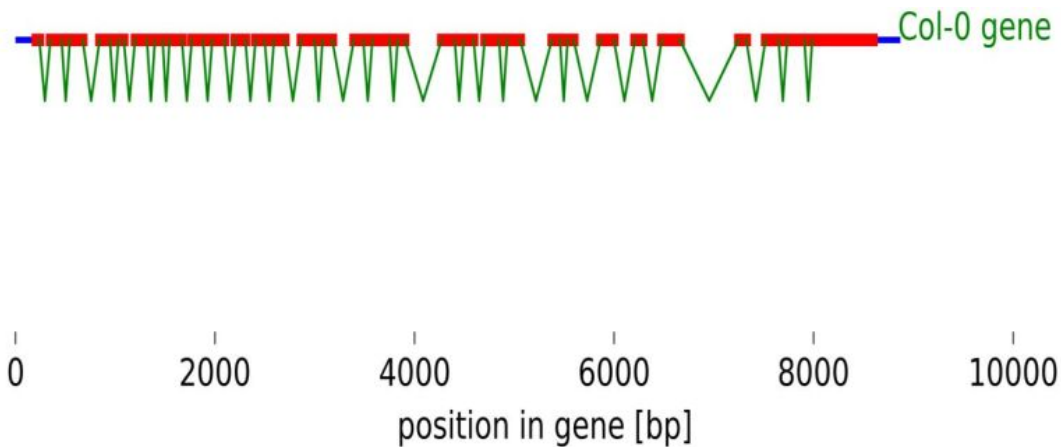


barplots.py generates
barplots at specific
positions by drawing a
normal line

(script is available in
course repository)

Gene structure plot

- gene_structure_plot.py generates visualizations of gene/transcript structures based on GFF annotations

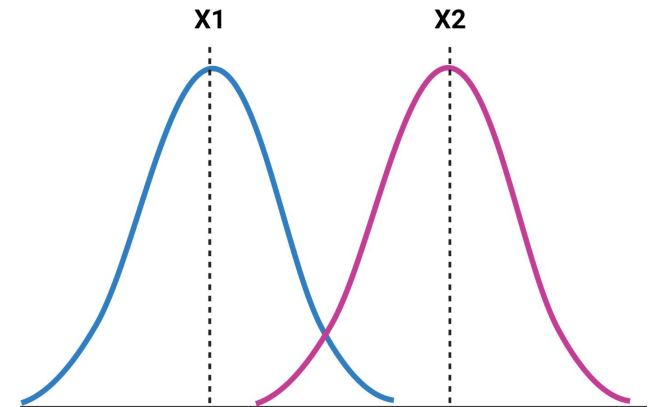


Exercises - Part6b

- 6.6) Construct a figure to illustrate the order and orientation of genes in the gum gene cluster of *Xanthomonas campestris* pv. *campestris*!
- 6.7) Save this figure in different file formats (png, jpg, pdf, svg)!

Statistics

- Compare observed sample against the expected distribution
- Check if two samples are derived from same distribution
- H_0 = samples were taken from same distribution
- H_0 can only be rejected or kept due to insufficient evidence against it
- H_0 can NEVER be confirmed



Shapiro-Wilk test

- Testing data set for normal distribution
- Important for decision about potential tests

```
from scipy import stats  
x = [1, 2, 3, 3, 3, 2, 1]  
stats.shapiro(x)
```

Correlation

- Pearson correlation coefficient is suitable for data following a normal distribution
- Spearman correlation coefficient is better if data distribution is unknown

```
from scipy import stats
x = [1,2,3,4,5]
y = [2,4,6,8,10]
r,p = stats.pearsonr(x,y)
r,p = stats.spearmanr(x,y)
```


t-test

- Samples need to show normal distribution
- Comparison of one sample against a reference value
- Comparison of two samples:
 - Paired samples (ttest_rel)
 - Unpaired samples (ttest_ind)

```
from scipy import stats
x = [1,2,3,4,5]
y = [4,6,8,10,11]
t,p = stats.ttest_ind(x,y) #independent samples
t,p = stats.ttest_rel(x,y) #paired samples
```

W-test

- Wilcoxon (W) test compares two paired samples
- Normal distribution is not required

```
from scipy import stats  
x = [1,2,3,4,5]  
y = [4,6,8,10,11]  
w,p = stats.wilcoxon(x,y)
```

U-test

- Comparison of unpaired samples
- Normal distribution is not required

```
from scipy import stats  
x = [1,2,3,4,5]  
y = [4,6,8,10,11]  
w,p = stats.mannwhitneyu(x,y)
```

Chi square test

- Comparison of an observation against an expectation
- Comparisons of two observations

```
from scipy import stats
obs = [1,2,3,4,5]
exp = [4,6,8,10,11]
x, p = stats.chisquare(obs,exp)
```

Exercises - Part6c

- 6.8) Construct a suitable visualization!
- 6.9) Analyze distribution and trends!
- 6.10) Apply statistical test to investigate difference!

HTML

- Construction of a HTML-based heatmap

gene ID	0H	4H	Salt	Heat	Inflorescence	leaf	Root	seedlings_HiK	seedlings_HiK2	Leaf_SSC
(p	0.02	0.0	0.0	0.0	0.0	0.0	4.33	1.44	1.2	0.0
(c	0.0	0.0	0.0	0.0	0.0	0.0	1.17	0.11	0.0	0.0
(c	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.11	0.4	0.0
p	1.76	29.23	14.65	6.11	203.75	265.2	0.83	199.33	199.7	54.4
o	0.0	0.0	0.0	0.01	1.38	0.0	0.0	0.0	0.1	0.0
(0.0	0.03	0.01	0.0	0.25	0.0	0.0	0.0	0.1	0.02
y	0.01	0.01	0.0	0.06	13.88	0.0	0.0	0.11	0.1	0.01
u	6.25	58.89	41.42	15.61	69.5	5.4	3.83	157.22	163.1	22.49
h	0.86	5.04	0.09	0.24	33.5	0.8	12.67	8.78	8.5	1.07
y	10.74	43.0	4.78	6.45	29.0	1.2	7.17	62.67	67.0	25.48
e	19.29	14.22	6.14	5.21	4.88	1.6	11.5	28.11	24.2	13.68
w	35.33	45.16	52.79	70.1	17.5	74.2	6.67	21.89	22.6	65.95
q	6.03	24.4	2.69	2.38	80.0	35.6	19.83	55.22	59.9	11.16
k	5.74	15.9	1.35	4.56	57.5	3.0	1.17	9.44	7.5	8.47
s	0.27	1.74	0.07	0.25	11.38	0.2	0.0	0.78	0.4	0.11
i	0.04	0.0	0.0	0.0	36.0	0.0	0.0	0.89	1.1	0.0
r	0.0	0.0	0.0	0.0	18.0	0.0	0.0	0.67	0.7	0.0
c	9.84	14.92	17.27	7.68	16.13	27.0	26.0	26.89	23.7	11.58
ev	17.99	9.41	7.94	8.45	11.38	21.2	13.83	19.22	19.7	14.81

HTML template

- construct_heatmap.py reads values from text file and prepares data structures to fill this template
- HTML document can be converted to PDF

```
1 <table border=1>
2 <tr>
3   {% for name in column_names %}
4     <th> {{ name }} </th>
5   {% endfor %}
6 </tr>
7 {% for gene in genes %}
8   <tr width="20px">
9     <th>{{ gene['name'] }}</th>
10    {% for value in gene['values'] %}
11      <td bgcolor={{value['color']}} width="20px"> <span title={{value['title']}}> <font color="black"> {{value['value']}} </font> </span></td>
12      <!-- one field contains the absolute value and has an corresponding background color -->
13    {% endfor %}
14  </tr>
15  {% endfor %}
16 </table>
```

different conditions/tissues

different genes/transcripts

precomputed color

value converted to test

Exercises - Part6d

- 6.11) Read data table and construct heatmap for the gene expression in HTML!
- 6.12) Add mouse-over effect to display functional gene annotation!

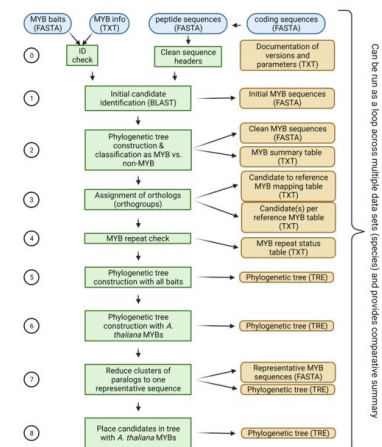
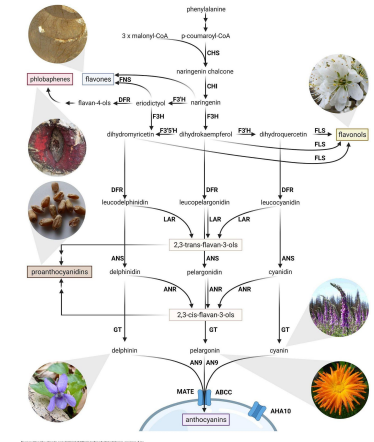
Looking for more opportunities to apply Python?

- Plant Biotechnology and Bioinformatics group is working on:

- Plant genomics
- Plant transcriptomics (RNA-seq)
- Specialized plant metabolites
- Synthetic biology
- Big data comparative studies
- Tool development

- Details: <https://www.tu-braunschweig.de/en/ifp/pbb>

- Web server: <https://pbb-tools.de/>



<https://doi.org/10.1186/s12864-022-08452-5>

Time for questions!

References (biological background of examples)

- Nd-1 genome assembly (Pucker *et al.*, 2016)
 - <https://doi.org/10.1371/journal.pone.0164321>
- Non-canonical splice sites (Pucker *et al.*, 2017)
 - <https://doi.org/10.1186/s13104-017-2985-y>
- *Croton tiglium* transcriptome assembly (Haak *et al.*, 2018)
 - <https://doi.org/10.3389/fmolb.2018.00062>
- Genome-wide non-canonical splice sites in plants (Pucker & Brockington, 2018)
 - <https://doi.org/10.1186/s12864-018-5360-z>
- Chromosome-level Nd-1 genome assembly (Pucker *et al.*, 2019)
 - <https://doi.org/10.1371/journal.pone.0216233>
- NAVIP (Baasner *et al.*, 2019)
 - <https://doi.org/10.1101/596718>